THE RELATION BETWEEN OLFACTORY RESPONSE AND RECEPTOR POPULATION IN THE BLOWFLY

V. G. DETHIER

Department of Biology, The Johns Hopkins University, Baltimore 18, Md.

In the course of an investigation to determine rejection thresholds of the blowfly, *Phormia regina* Meigen, for the vapors of homologous alcohols it became desirable to ascertain whether or not the response elicited depended entirely upon stimulation of the olfactory sense, as commonly believed, or represented instead a less specific action by way of a common chemical sense. In order to check this point it was necessary to measure accurately the threshold concentration for some test substance in the vapor phase and then to follow quantitatively changes in threshold value as different areas suspected of bearing olfactory receptors were extirpated. Execution of this plan showed not only that rejection was mediated almost entirely through the olfactory sense, but it yielded in addition some unexpected information relative to the comparative sensitivity of the different olfactory receptor fields and the importance of bilateral versus unilateral stimulation. This last is of particular interest if one is to give due consideration to the possibility of summing and to the relative contributions of the peripheral and central components of the nervous system to those aspects of behavior which depend initially upon stimulation of the sensory system.

Methods and Results

By analogy with other insects, the principal areas to be suspected of bearing olfactory receptors are the antennae, the labial palpi, and the labellum. Frings (1941) had shown by conditioning experiments that the olfactory sense of a related species, *Cynomyia cadaverina* Desvoidy, is resident in the antennae and labellum. Experiments by Dethier and Chadwick (1947) in another connection had shown that the response of *Phormia* to the odors of many organic compounds is abolished when the antennae and labellum are removed. The present investigation was designed to establish these results on a quantitative basis and to assess the contribution of the several sensory areas to the rejection response. Pentanol was selected as a test compound because of the decisive manner in which flies responded to it and the exactness with which a threshold value could be measured. The technique of threshold measurement has been described in detail in an earlier communication (Dethier and Yost, 1952).

For the present study, measurements of response were made with normal animals and animals from which head appendages had been removed by cutting, singly or in various combinations. Instead of varying the concentrations and determining what strength of stimulus was required to elicit 50% response (median rejection threshold) under each experimental condition, the simpler procedure was adopted of selecting two concentrations and determining the change in the per cent of the

TABLE I

Response of normal and operated Phormia to the vapor of pentanol

Receptor areas remaining	Mean % rejection (1)	Mean % rejection (2)	t value	Probability	Significance of difference
Tested at 5.0×10^{-6} *M*					
(1) All (normal) (2) Blank (pure air: no stimulus)	93.3	13.0	23.0	<0.01	+
(1) All except 1 ant. (2) Blank	69.3	11.8	11.3	<0.01	+
(1) Labellum and palpi (2) Blank	47.2	13.8	5.2	<0.01	+
(1) Palpi (2) Blank	34.5	12.7	3.5	<0.01	+
(1) None (2) Blank	15.7	3.0	2.8	0.025	slight
(1) All (normal) (2) All except 1 ant.	93.3	69.3	4.2	<0.01	+
(1) All except 1 ant. (2) Labellum and palpi	69.3	47.2	2.8	0.014	slight
(1) Labellum and palpi (2) None	47.2	15.7	4.4	<0.01	+
(1) Labellum and palpi (2) Palpi	47.2	34.5	1.6	0.15	–
(1) Palpi (2) None	34.5	15.7	2.6	0.02	slight
Tested at 1.6×10^{-6} *M*					
(1) All (normal) (2) Blank	32.3	6.6	4.9	<0.01	+
(1) Labellum and palpi (2) Blank	11.3	11.2	0.012	>0.9	–
(1) None (2) Blank	10.9	10.0	0.21	0.84	–
(1) All (normal) (2) Labellum and palpi	32.3	11.3	2.8	0.017	slight
(1) Labellum and palpi (2) None	11.3	10.9	0.052	>0.9	–

population responding as the various appendages were removed. Since the relation between the per cent of any population responding and the concentration to which it responds is known (the distribution is normal with reference to the logarithm of concentration), this method of approach is justified. The two concentrations selected were 5.0×10^{-6} *M* pentanol, to which approximately 90% of the population normally responds, and 1.6×10^{-6} *M*, to which approximately 25% normally responds. The median is 2.2×10^{-6} *M*.

The results, together with tests of significance, are listed in Table I.

Discussion

Three points for consideration emerge from an inspection of the foregoing data. These relate to the location of receptors, the differential sensitivity of receptor fields, and the difference between unilateral and bilateral thresholds.

When the higher concentration of pentanol is employed as a test stimulus, response in the form of rejection by the animal is abolished only when all of the head appendages (*i.e.,* antennae, palpi, and labellum) are destroyed. On statistical grounds there is still a slight possibility of a retention of some residual sensitivity even then to this concentration (see Table I, "Tested at 5.0×10^{-6} *M*, No Receptors Remaining"), but the degree of response is very low. The reduction of response is most marked following removal of the antennae. No difference in response could be detected between animals possessing labella and palpi only and those possessing palpi only. Therefore, receptors sensitive to the lower concentrations of pentanol are presumed to be present on the antennae and palpi.

This conclusion is in conflict with those previously reported by Dethier and Chadwick (1947) and Frings (1941). Part of the discrepancy may be resolved by giving attention to the problem of stimulus concentration. When a weak stimulus is employed for testing, response is completely abolished following removal of antennae alone (Table I, "Tested at 1.6×10^{-6} *M*, Labellum and Palpi Remaining"). In the earlier experiments with *Phormia,* where the responses of normal versus antennectomized-labellectomized flies were compared, it had been observed that the latter failed to respond to the odors of solutions employed in stimulating tarsal chemoreceptors. The test odors, although not quantitatively controlled at that time, were of low concentration, and a repetition of the experiments has subsequently shown that antennectomy alone is sufficient to prevent the olfactory response of flies under those conditions of testing.

The data for *Phormia* and those obtained by Frings with *Cynomyia cadaverina* could be reconciled by an explanation which would take advantage of the species difference, but this is admittedly unsatisfactory. On the other hand, it is conceivable that the differential sensitivity of the various appendages in the two species is not identical. If receptors sensitive to stimulation by compounds in the vapor phase were considered to be present on all three sets of appendages, the comparative sensitivity in the two species might be antennae > labellum > palpi in *Cynomyia* and antennae > palpi > labellum in *Phormia.* If the stimulus were of moderate to low concentration, only the antennae and labellum would be involved in the first species and the antennae and palpi in the second. Knowledge of such relationships would emerge only after a careful study of the entire concentration range. The fact remains, nonetheless, that *Phormia* is repelled by low concentrations of gaseous substances only when both antennae are intact and by high concentrations only when the antennae or palpi are intact. Therefore repellents in the vapor state may be presumed to be acting on olfactory receptors.

Differences between responses at high and low concentrations of pentanol show quite forcefully that all receptor fields are not equally sensitive and that the field on the mouthparts is much less so than that on the antennae. Additional confirmation for this last conclusion is seen in the degree to which response is reduced following removal of different appendages. The greatest reduction follows bilateral antennectomy. This confirms what has been known for some time, that the antennae of insects are generally sensitive to lower concentrations of odors than other receptor fields. Whether the difference is to be ascribed to a difference in the number of receptors involved, in their intrinsic thresholds, or in their central nervous connections cannot be known at this time.

The possibility of a dependence of total response upon activation of a threshold number of receptors has not been carefully examined in olfaction. The phenomenon has been investigated to some degree for the humidity sense. The suggestion that a threshold number of receptors is required for a response to humidity changes was first demonstrated by Pielou (1940) on evidence gathered with the mealworm, *Tenebrio molitor*. Detailed confirmation was provided by the experiments of Roth and Willis (1951a) which showed that the per cent of response of a population of two species of *Tribolium* was closely correlated with the number of sensilla basiconica remaining on each individual after surgical operation. As the number was progressively decreased the per cent response to humidity differences correspondingly decreased.

The present data on olfactory response to high concentrations of pentanol permit an extension of the analysis of the relation between receptor number and threshold of response. The existence of such a relation is indicated by the significant difference in response between those flies possessing both antennae and those with a single antenna. Observation of individual flies, plus the fact that the response of flies with a single antenna could be made to match the response of normal flies by adjustment of the stimulating concentration, indicated that results could not be attributed to interference with locomotory behavior.

If one makes the assumption that x number of receptors must be activated to insure a response, then at the median threshold value 50% of the flies have x or more receptors acting. The probability at this concentration that a fly (x or more receptors) will be in the half of the population which is responding is 50%. This probability can be increased either by increasing the concentration or by increasing the number of available receptors (n). If n is doubled, the probability of a response increases. This increase can be calculated from the expression $1\text{-}q^2$ where q is equivalent to the per cent not acting at the median concentration. Accordingly, the concentration which elicits a response from 50% of the flies possessing a single antenna should elicit a 75% response from the flies possessing both antennae if there is no interaction between the two antennae.

Although the concentration chosen to test this hypothesis was the 90% level rather than the 50% level, use of the expression $1\text{-}q^2$ still yields the desired results. Application of the test to the data shows that the decrease in threshold with two antennae as opposed to one can be accounted for satisfactorily on a probability basis.

Since the probability of a response can be increased (reflected as a decrease in threshold concentration) either by increasing the receptor number or by increasing the concentration and holding the receptor number constant, it may indeed be concluded that some relation exists between concentration and number of receptors activated. Furthermore, it can be said that as the concentration is increased a larger number of receptors is activated. Beyond this one cannot go on the basis of these data.

Table II

Effects of unilateral and bilateral operations in the responses of beetles given a choice between 0% and 100% R.H. at 27° C.
(Data from Roth and Willis, 1951b)

Species	Receptor areas remaining on antenna	% response
Rhyzopertha dominica (thin-walled sensilla present on segments 8 to 10)	Segments 1–10 on both (normal)	76 ± 2.7
	Segments 1–10 on one; Segments 1–9 on other	69 ± 3.0
	Segments 1–9 on both	56 ± 3.4
	Segments 1–10 on one; Segments 1–7 on other	61 ± 2.9
	Segments 1–7 on both	−0.1 ± 7.0
Latheticus oryzae (thin-walled sensilla present on segments 7 to 11)	Segments 1–11 on both (normal)	87 ± 3.1
	Segments 1–11 on one; Segments 1–10 on other	73 ± 3.2
	Segments 1–10 on both	46 ± 2.7
Tribolium castaneum (thin-walled sensilla present on segments 9 to 11)	Segments 1–11 on both (normal)	80 ± 1.0
	Segments 1–9 on both	75 ± 2.9
	Segments 1–9 on one; Segments 1–8 on other	78 ± 3.6
	Segments 1–8 on both	31 ± 5.4
Tribolium confusum (thin-walled sensilla present on segments 7 to 11)	Segments 1–11 on both (normal)	86 ± 1.7
	Segments 1–11 on one; 0 segments on other	76 ± 3.0
	0 antennae	−0.2 ± 2.2
	Segments 1–7 on both	23 ± 1.6
	Segments 1–7 on one; Segments 1–6 on other	12 ± 3.9
	Segments 1–6 on both	5 ± 4.1
	Segments 1–11 on one; Segments 1–6 on other	75 ± 2.1
	Segments 1–6 on both	5 ± 4.1

Rise of threshold with unilateral extirpation has not been emphasized before, but it is implicit in many recorded data. It is seen, for example, in the humidity responses of many beetles. Several selected data of Roth and Willis (1951b) show it clearly (Table II). Here the response is always (with the one exception in the case of *Tribolium castaneum*) reduced when one antenna or portion thereof is removed. The response is further diminished when corresponding areas on the remaining antenna are removed. In none of these cases, nor in the olfactory data presented, does the experimental value of response to bilateral stimulation exceed that for unilateral stimulation by a value greater than that which can be accounted for by statistical bias. Consequently, it can be stated that there is here no evidence for contralateral neural summation (*cf.* Dethier, 1950). However, when only one or two concentrations rather than a complete frequency distribution have been investigated, it is not possible to analyze the situation fully. Nor can one apply a rigorous test of significance to the difference between the experimental value for bilateral stimulation and the calculated value and consequently settle the question of summation with any great degree of certainty. This follows in part because the Chi square test is inaccurate when small numbers are used and only one degree of freedom is involved.

Since decrease of unilateral threshold over bilateral threshold has not been recorded as occurring commonly among animals (the difference between monaural and binaural thresholds, and monocular and binocular thresholds in man being two of the better known examples), the phenomenon is worthy of closer scrutiny. Additional experiments with the tarsal chemoreceptors, which are more amenable to analysis, are in progress to settle this point. At the moment it would appear that the results reported here do not indicate the existence of true neural summation but instead what has been termed supplementation by Smith and Licklider (1949). Nonetheless, insofar as interaction exists between bilaterally placed receptor fields, it remains from the point of view of the integrated organism a behavioral summation of no little importance.

Summary

1. Measurements have been made of thresholds of rejection of two concentrations of pentanol vapor by normal blowflies and those from which various head appendages have been removed. The concentrations selected were 5.0×10^{-6} *M* pentanol, to which 90% of the population normally responds, and 1.6×10^{-6} *M*, to which approximately 25% of the population normally responds.

2. The results show that the response elicited depends upon stimulation of specialized receptors present on the antennae and palpi and hence cannot be attributed to the action of a common chemical sense. Repellents in the gas phase undoubtedly act as olfactory stimuli. Different receptor fields function at different levels of sensitivity. The antennae are the most sensitive and the various mouthparts less so. There is also a relation between threshold and the number of receptors functioning. Insects possessing both antennae respond to lower concentrations of odor than those bearing only one. This indicates that the two sides of the body are acting in concert. Whether it represents true neural contralateral summation or is based upon statistical bias cannot yet be decided with certainty although the data strongly suggest the latter. In either case, from the point of view of coordinated behavior it illustrates the manner in which bilaterally placed receptor fields may operate as a unit.

LITERATURE CITED

Dethier, V. G., 1950. Central summation following contralateral stimulation of tarsal chemoreptors. *Fed. Proc.*, **9**: 31–32.

Dethier, V. G., and L. E. Chadwick, 1947. Rejection thresholds of the blowfly for a series of aliphatic alcohols. *J. Gen. Physiol.*, **30**: 247–253.

Dethier, V. G., and M. T. Yost, 1952. Olfactory stimulation of blowflies by homologous alcohols. *J. Gen. Physiol.* (in press).

Frings, H., 1941. The loci of olfactory end-organs in the blowfly, *Cynomyia cadaverina* Desvoidy. *J. Exp. Zool.*, **88**: 65–93.

Pielou, D. P., 1940. The humidity behaviour of the mealworm beetle, *Tenebrio molitor* L. II. The humidity receptors. *J. Exp. Biol.*, **17**: 295–306.

Roth, L. M., and E. R. Willis, 1951a. Hygroreceptors in adults of Tribolium (Coleoptera, Tenebrionidae). *J. Exp. Zool.*, **116**: 527–570.

Roth, L. M., and E. R. Willis, 1951b. Hygroreceptors in Coleoptera. *J. Exp. Zool.*, **117**: 451–488.

Smith, M. H., and J. C. R. Licklider, 1949. Statistical bias in comparisons of monaural and binaural thresholds: binaural summation or binaural supplementation. *Psychol Bull.*, **46**: 278–284.